Product No. C 5713
Monoclonal Anti-Human CD62P (P-Selectin)
Purified Mouse Immunoglobulin
Clone AK 4

Monoclonal Anti-Human CD62P (P-Selectin) (mouse IgG1 isotype) is derived from the AK 4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Purified human platelet glycoprotein membrane fraction (100-200 kD) immunodepleted of GP-IIb-IIa (CD41) was used as the immunogen.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The product is purified using protein A and provided 0.2 µm-filtered in 0.01 M phosphate buffered saline pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Monoclonal Anti-Human CD62P (P-Selectin) recognizes the human CD62P antigen expressed by activated platelets and endothelial cells.

Description

P-selectin (CD62-P, GMP-140, LECAM-3, PADGEM) is a member of the LEC-CAM family of adhesion receptors that recognize specific carbohydrate ligands and mediate an early step in the interaction of leucocytes with endothelium, megakaryocytes and platelets. It is a cell surface 140-150 kD glycoprotein, the extracellular region of which contains NH_2-terminal C-type lectin domain, followed by a homologous EGF-like domain and nine short consensus repeats (SCR).[2] P-selectin is an α granule membrane glycoprotein which is rapidly translocated to the plasma membrane of platelets in response to various inflammatory and thromobogenic agents. It is also located in the endothelial Weibel-Palade bodies and on the surface of activated endothelial cells. A circulating soluble form of P-selectin is found in the blood.[3] Levels of soluble P-selectin in biological fluids may be elevated in patients with various pathological conditions. P-selectin mediates transient neutrophil adhesion to thrombin-stimulated endothelial cells membrane ('rolling' at physiological shear stress) and the binding of activated platelets to myeloid cells. It binds to the carbohydrate sialyl-Lewis (CD15s) on neutrophils and to sulfated galactosyl ceramides on neutrophils and tumor cells. Binding of P-selectin to its ligand is Ca^{2+} dependent.[4] Monoclonal Anti-Human CD62P (P-selectin) partially inhibits the P-selectin-mediated rosetting of HL-6 cells by thrombin-activated platelets.[5]

Uses

Monoclonal Anti-Human CD62P (P-selectin) may be used for identification of activated platelets and endothelial cells by flow cytometry and immunocytochemistry. It is also useful in detecting soluble P-selectin.

Performance

When assayed by flow cytometric analysis using 5 µl of the antibody to stain 1 x 10^6 platelets, a fluorescence intensity similar to that obtained with saturating monoclonal antibody levels is observed.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage

Store at 2-8°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

* Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

References

1. Skinner, M., et al., Biochem. Biophys. Res. Commun., **164**, 1373 (1989).
2. Johnston, G., et al., Cell, **56**, 1033 (1989).
3. Dunlop, L., et al., J. Exp. Med. **175**, 1147 (1992).
4. Tedder, T., et al., FASEB J., **9**, 866 (1995).
5. Skinner, M., et al., J. Biol. Chem. **266**, 5371 (1991).

Procedure for Indirect Immunofluorescence Staining of Human Platelets

Reagents and Materials Needed but not Supplied

1. Human peripheral blood platelets isolated from platelet rich plasma.
2. Diluent: 10 mM PBS, pH 7.4, with 1% BSA and 0.1% NaN_3.
3. ADP Reagent (Sigma Product No. 885-3).
4. 1% Paraformaldehyde solution in PBS.
5. FITC or PE conjugated anti-mouse secondary antibody at recommended working dilution in diluent (Sigma Product No. F 2653 is recommended). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
6. Isotype matched non-specific mouse immunoglobulin (negative control, Sigma Product No. M 5284).
7. 12 x 75 mm test tubes.
8. Adjustable micropipet.
9. Centrifuge.
10. Counting chamber.
11. Trypan blue (Sigma Product No. T 0776), 0.2% in 10 mM PBS, pH 7.4 (Sigma Product No. P 4417).
12. 2% paraformaldehyde in PBS.
13. Flow cytometer.

Procedure

1. Rapidly prepare washed platelet suspension in diluent. Adjust cell suspension to 1-3 x 10^7 viable platelets/ml.
2. Centrifuge at 1,000 x g for 15 min at room temp and remove supernatant by careful aspiration.
3. Activate platelet pellet with 50 µl of reconstituted ADP reagent. Incubate at room temp. for 5 minutes.
4. Stop activation by adding 1 ml cold 1% paraformaldehyde solution in PBS. Let for at least 1 hour at 2-8°C. Add 3 ml diluent and centrifuge at 1,000 x g, for 10 minutes.
5. Remove supernatant by careful aspiration and resuspened platelets in diluent to 1-3 x 10^7 cell/ml.
6. Add 100 µl or 1-3 x 10^6 platelets per tube.
7. Add 5 µl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the platelets at 2-8°C for 60 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl diluent in place of monoclonal antibody followed by steps 8-13 but replacing secondary antibody in step 11 with 100 µl of diluent.
   b. Negative control: 5 µl isotype- matched, non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 8-13.
   c. Negative control: 5 µl of diluent in place of monoclonal antibody followed by steps 8-13.
8. After 60 minutes, add 3 ml of diluent to all tubes.
9. Pellet platelets by at 1,000 x g, for 10 minutes.
10. Remove supernatant by careful aspiration.
11. Resuspend the platelets in 100 µl of the fluorochrome conjugated secondary antibody at the recommended dilution or in diluent (for autofluorescence control 7.a). Incubate at room temperature or 2-8°C for 30 minutes. Protect from light at this and all subsequent steps.
12. Add 3 ml diluent. Centrifuge and wash as in steps 9 and 10.
13. Resuspend platelets in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and analyze in a flow cytometer according to manufacturer's instructions.

Notes

1. Platelet activation may increase (e.g., CD62P), decrease (e.g., CD31) or have no effect on marker expression.
2. Demonstration of platelet activation effects on antigen expression is dependent on immediate processing. Some activation of resting platelets may occur during preparation even without added activators.
3. A positive staining control: 5 µl Anti-Human CD61 (Sigma Product No. C 4321), Anti-Human CD36 (Sigma Product No. C 4679) or Anti-Human CD42a (Sigma Product No. C 4196) at their respective optimal dilution.

Quality Control

It is advisable to run the appropriate negative controls since they establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.